SECTION 6

SAMPLE PROCESSING

6.1 Sieving

6.1.1 Samples collected with grabs, coring devices, and artificial substrates contain varying amounts of finely divided materials such as decomposed organic material, silts, clays, and fine sand. To reduce sample volume and expedite sample processing in the laboratory, these fines should be removed in the field by passing the sample through a U.S. Standard No. 30 sieve. Sieves may be commercial models or homemade sieves framed with wood or metal. Floating sieves with wooden frames reduce the danger of accidental loss of both sieve and sample when working over the side of a boat in deep waters. A sieve should contain no cracks or crevices in which small organisms can become lodged.

6.1.2 Sampling efficiency is increased by using sieves with smaller mesh openings (Mason et al., 1975; Barber and Kevern, 1974; and Zelt and Clifford, 1972). However, use of the smaller mesh size does not have an appreciable effect on the eutrophic classification based on common biotic indices. Precision based on coefficient of variation (CV) increased with smaller mesh size (Mason et al., 1975). Usually the increased length of time required to use the smaller mesh sieve sizes is not compensated for by the increased accuracy of results (Hummon, 1981). Also, organisms passing through the U.S. Standard No. 30 sieve are not macroinvertebrates by definition. (See Section 1, Introduction).

6.1.3 If at all possible, sieving should be done in the field immediately after the sample is collected and the captured organisms are still alive, but time can often be saved by returning to the laboratory with the samples unsieved and doing the sieving with a mechanical device such as the elutriation apparatus described by Worswick and Barbour (1974). If the sample is likely to include tubificid worms, leeches, or Turbellaria, a few representative specimens of each should be picked out before sieving and fixed in 10% buffered formalin or transported live to the laboratory for fixing or immediate identification. Once preserved, many organisms become quite fragile and if subjected to sieving will be broken up, lost, or rendered unidentifiable. Great care should be taken in sieving preserved samples containing mayflies, stoneflies and worms to reduce breaking the specimens or otherwise damaging body parts necessary for identification.

6.1.4 Sieving may be accomplished by one of several techniques depending upon the preference of the biologist. In one method, the sample is placed directly into a sieve and the sieve is then partially submerged in water and agitated until all fine materials have passed through. The sieve is agitated, preferably in a large tub of water but sieving may be done over the side of the boat if care is taken not to spill the sample. A variation of this technique is to place the original sample in a tub or bucket, add screened water, stir, and pour the resulting slurry through a U.S. Standard No. 30 sieve. Only a moderate amount of agitation is required to completely

clean the sample. Since this method requires considerably less effort, most biologists may prefer it. A sieve bucket (Fig.11) described by Hiltunen (1983) for use in the Great Lakes works well under most conditions and allows the sample to be sieved while the boat is under way to the next sampling site. The cycle sieve described by Mason (1976) works well in calm weather from a small boat but is cumbersome and impractical for use from large boats, bridges or other such structures. In all of the above methods, remove, carefully clean, and discard all the larger pieces of debris and rocks from the sample before stirring or agitating.

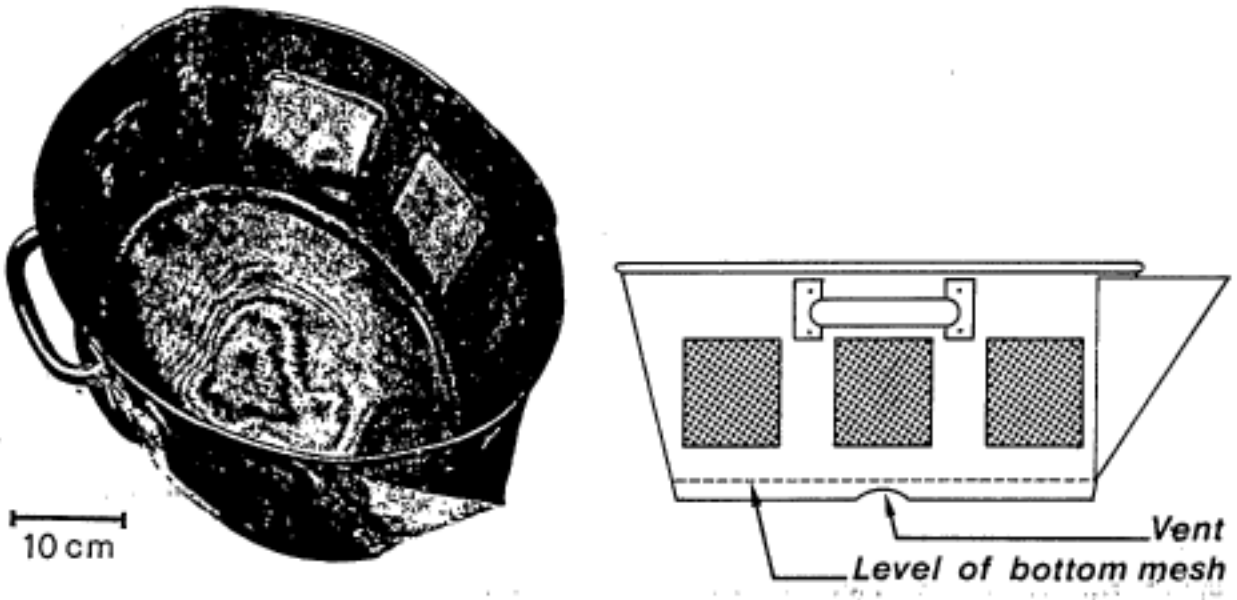

Figure 11. Great Lakes sieve bucket (From Hiltunen, 1983).

6.1.5 Artificial substrate samplers are placed intact into a bucket or tub of screened water and dismantled. Each individual piece of substrate is rinsed, gently but thoroughly cleaned under water with a soft brush such as a soft bristled toothbrush, examined visually, and laid aside. The water in the bucket or tub is then poured through a U.S. Standard No. 30 sieve to remove the fines. After most of the fines are washed from the sample, the organisms are left scattered over the surface of the screen. These organisms can be picked from the screen with forceps and placed in the sample container. A faster method is to concentrate them at one edge of the sieve by gently swirling the sieve in a little water, then tilting the sieve over a wide-mouth jar and gently backflush the organisms into the jar with water from a wash bottle directed through the screen.

6.1.6 Another way to separate the organisms from the detritus is the flotation method in which a concentrated aqueous solution of sugar, salt, or other chemical is poured over the sample in the tub or bucket causing the animals to float up out of the detritus due to the difference in the specific

gravity of the animals and solution. The organisms can then be poured or scooped into the sample container with a sieve spoon. Some organisms, such as clams and snails, must still be hand picked from the debris because they are too heavy to float. Two or three lbs. of sugar per gallon of water makes a good flotation solution (Anderson, 1959).

6.1.7 When drift net or Surber-type samplers are being used, it is usually possible to empty the bag directly into a white bottom enamel pan or small bucket and hand pick the organisms into a sample container filled three-fourths full of preservative.

6.1.8 Although the U.S. Standard No. 30 (600 μm) sieve is also commonly used in marine studies, some investigators (Grassle et al. 1985) have chosen to use a 300 μm sieve in order to more efficiently sample smaller and juvenile macrofauna. This practice requires more time and taxonomic expertise. The 600 μm sieve is usually adequate since the vast majority of macrofaunal biomass and production is associated with larger forms.

6.1.8.1 For marine work the use of more than one sieve in series, one on top of the other, allows benthic communities to be fractionated by size allowing comparisons of community size distributions between stations and over time. Commonly used sieve sizes are 300 μm, 500 μm, 600 μm, 1 mm, and 2 mm.

6.1.8.2 Sieving marine samples should be done by rinsing organisms with a gentle spray of water to minimize mechanical damage to the organisms. Direct heavy jets of water should not be used and an elutriate procedure that ensures that the major source of water is from the bottom of the sieves is recommended. Water used in sieving should be obtained from the sample site whenever possible. Fresh water should never be used to sieve unpreserved marine fauna because of osmotic effects that cause cell bursting.

6.2 Preservation and Fixation

6.2.1 All samples collected in the field should be preserved in 70-80% ethyl alcohol (ethanol), but ideally, and for ease in identification, representative specimens of leeches, aquatic oligochaetes, and other soft bodied organisms, if time permits, should first be fixed in 10% formalin to fix the tissue. After fixation (about 10 minutes), depending on size and number of organisms, or after returning to the laboratory, they may be preserved in 70-80% ethanol. This process should aid in their identification (see Section 6.5.4. and 6.5.5). Because wash water is contained in the sieved material, the stock preservative solution added to the sample should be over-strength (90%) so that the final solution will be sufficient to preserve the organisms. Grab samples collected from lakes, the muddy bottoms of large rivers, estuaries and oceans are often fixed and preserved in ten percent buffered formalin because they contain many worms which are difficult to identify after being preserved in ethanol. Formalin should be buffered to a neutral or slightly alkaline level with borax.

6.2.2 Since leeches dropped alive into preservatives such as 70-80% ethanol or 10% formalin solution contract strongly, some diagnostic features used

for species identification may be difficult to determine by the inexperience. Ideally, specimens should first be narcotized by direct placement into carbonated water, fixed in 10% formalin, and preserved in 70-80% ethanol. If this procedure is inconvenient in the field, the specimens should be preserved directly in 70-80% ethanol. Most specimens still can be identified to species but might take a little longer than usual. Additional collecting, narcotizing, and processing techniques can be found in Klemm (1982, 1985).

6.2.3 Turbellarians that require identification to species should be transported to the laboratory alive in a small amount of water (Pennak, 1978, 1989).

6.2.4 Although not always necessary, species identifications are easier and morphometric analyses are facilitated if marine organisms are relaxed after sieving and prior to fixation and preservation. Organisms to be relaxed are transferred from sieves to a fine mesh (approximately 100 μm) bag and placed in a solution of magnesium chloride (approximately 75 g/l) for about 10 minutes. The organisms may then be fixed and preserved.

6.2.4.1 A 10% (by weight) formalin solution is most commonly used to fix and preserve marine samples. The solution is buffered to keep the dissolution of molluskan shells to a minimum.

6.2.4.2 Because formaldehyde is a carcinogen, and because some individuals develop severe sensitivities to formaldehyde over time, some researchers prefer to transfer samples from formalin to ethanol for preservation. This is acceptable if samples are only to be used to do taxonomic studies. However, biomass measurements should not be done on samples preserved in ethanol. Although weight loss due to preservation in formalin is significant (10-20%) (Mills et al., 1982; Schram et al., 1981; Williams and Robins 1982), weight loss due to preservation in ethanol is greater.

6.2.5 Sample containers used for holding preserved samples should be large enough so that they are not over one-half full of the washed sample before the preservative is added. Quart or liter sized jars are adequate for most samples collected with artificial substrate, drift net, or square-foot type samplers, but two or more jars may be needed for a grab sample depending on the amount of detrital material mixed with the sample. Hand picked specimens are usually preserved by placing them directly into small screw-cap vials filled with 70-80% ethanol.

6.2.6 If the samples are not sorted within two or three weeks after collecting, the preservative should be poured off and replaced with fresh preservative for permanent storage (Cairns and Dickson, 1971).

6.2.7 After sorting and/or identification most macroinvertebrates should be stored in a solution of 70-80% ethanol and 5% glycerine in vials sealed with tightly fitting rubber stoppers. If screw-cap vials are used, they should be submerged in 70-80% ethanol in a larger container and should be checked yearly to replace alcohol lost because of evaporation or Teflon tape can be used to secure the screw-caps to prevent evaporation.

6.3 Labelling and Record Keeping

6.3.1 All sample containers must be labeled in the field immediately upon collection. Sample labels made of water-resistant paper should be placed inside each sample container. Write all information on the label with a soft-lead pencil or waterproof ink. Where the volume of sample is so great that several containers are needed, additional external labels with sample number and notations such as 1 of 2, 2 of 2, etc. are helpful for identifying the sample containers when the samples are logged in at the laboratory. All labels must include a sample identification number which corresponds to the number entered in the field notebook for that sample, the sampling date, water body and location from which the sample was collected, and the name of the collector. In addition to the information on the label, the field notebook should include the sampling method, weather, substrate characteristics, depth, and any other physical or environmental conditions noted.

6.3.2 Marine sample data sheets should include date of collection, time of day, station number, geographic coordinates, replicate number, core penetration depth, and the identification number and final storage location of each sample. These data sheets should also include space for comments on the visual appearance of each sample (e.g., obvious tubes or burrows, presence or absence of a surface flocculent layer, sediment color, apparent depth of the redox-potential discontinuity, etc.); ancillary data such as water temperature, salinity, secchi disk visibility, vertical profiles of dissolved oxygen; and other data potentially useful in the interpretations of benthic community data.

6.3.3 As soon as possible after returning to the laboratory, each sample should be assigned an ID number in sequence. This number identifies the sample in a bound ledger where all the information from the field label and field notebook are recorded for permanent record. The sample ID number must also be placed prominently on the sample container before storing so that it can be identified when needed. This sample ID number should be placed on all specimen vials, microscope slides, and other items connected with the sample.

6.4 Sorting and Subsampling

6.4.1 Sorting

6.4.1.1 Sort through the samples by hand in the laboratory using a low power (2X) scanning lens or a stereomicroscope. Place one or two tablespoonfuls of the sample in a white enamel pan (size 25 X 40 X 5 cm) filled about one-third full of water. Usually small insects and worms will float free of most of the debris when ethanol-preserved samples are transferred to the pan. These floating organisms should be removed before they soak up water and sink. They can be skimmed off with a sieve spoon or poured off. Addition of about one tablespoon full of sugar and stirring the sample will cause most of the other organisms to float free. Flotation in formalin-preserved samples is accomplished by adding sugar slowly to raise the specific gravity to 1.12 (Pask and Costa, 1971). Numerous other techniques have been proposed to aid recovery of the organisms from the sample debris, including solutions

of magnesium sulphate, D-mannitol, calcium chloride or sodium chloride; electricity; bubbling air through samples in a tube, etc. The efficacy of these techniques is affected both by the characteristics of the substrate material and the types of organisms present (Flannagan, 1973). Regardless of the sorting method used, heavy organisms such as clams and snails will not float and will have to be picked out with forceps.

6.4.1.2 Various staining methods have been devised to help speed the sorting process (Williams and Williams, 1974). Staining samples in the field with either rose bengal or phloxine B at a concentration of 100 g/L of ethanol or formalin significantly reduces sorting time for benthic samples (Mason and Yevich, 1967). Should the stain interfere with identifications where color patterns or internal organs must be examined, the stain can be removed by placing the organisms in 95% ethanol over night.

6.4.1.3 As soon as the sample is sorted, make note in the log book, including the date and the initials of the person who sorted the sample. It is often advisable to ask a co-worker to check the sample debris before discarding to be certain no organisms were overlooked. The organisms may be sorted and transferred to watch glasses or petri dishes for immediate identification and counting, or stored in vials for future identification.

6.4.2 Subsampling

6.4.2.1 Analysis time for samples containing large numbers of organisms can be substantially reduced if the samples are subdivided before sorting. There are several methods for subdividing the samples and each method has its advantages and disadvantages.

6.4.2.2 Welch (1948) described a method that has been used successfully for many years. The sample is thoroughly mixed and distributed evenly over the bottom of a shallow white-bottom pan. A divider, delineating one-quarter sections, is placed in the tray and one quarter or two opposite quarters are sorted.

6.4.2.3 An air driven subsampler (Figure 12) was described by Wrona et al. (1982) and modified by the State of Maine Department of Environmental Protection (Susan Davies, Personal communication). The sample is placed in a Imhoff-type settling cone that is filled with water to a total volume of one liter. The sample is gently agitated for two to five minutes by use of an air stone sealed into the bottom and connected to an air supply. One-quarter of the sample is removed with a wide-mouth 50 mL dipper or test tube in five aliquots and combined in a white-bottom pan for hand sorting. If less than 100 organisms are present in the one-quarter subsample, additional one-quarter subsamples are removed until the subsample contains at least 100 organisms. Large or heavy organisms that cannot be suspended by agitating the water are sorted and counted separately.

6.4.2.4 The Rapid Bioassessment Protocols II and III (Plafkin et al., 1989) use a modification of a subsampling method described by Hilsenhoff (1987).

All large detrital material (leaves, twigs, etc.) are rinsed, visually

inspected for organisms, and discarded. The sample is then poured into a white-bottom pan that has been marked with a grid pattern of 5-cm squares. Grids are randomly selected and all the organisms in the selected grids are picked in succession until approximately 100 organisms have been removed from the sample. All the organisms in the grid that contains the 100th organism are picked once that grid is started. Before using this method, live organisms should be narcotized with club soda or nicotine before sorting so they will not move from square to square.

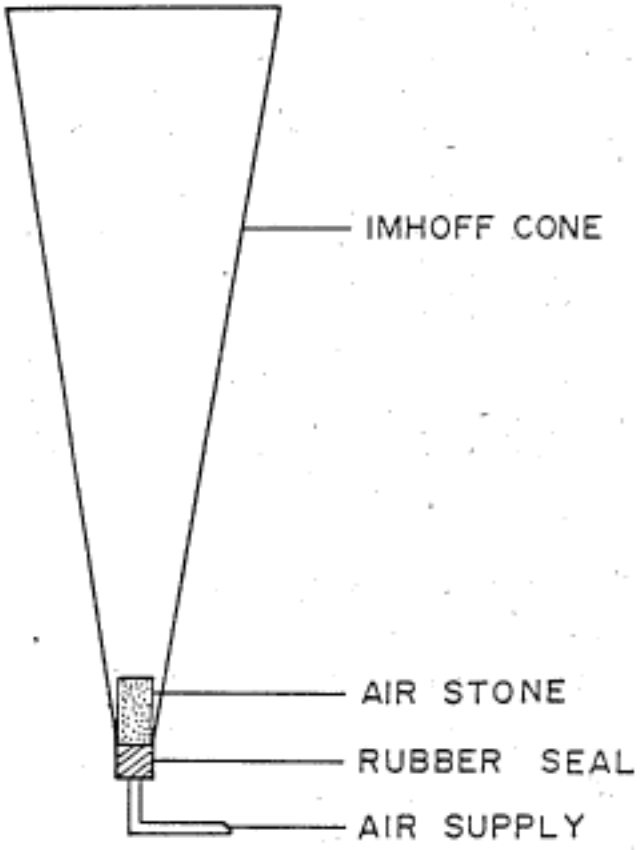

Figure 12. Imhoff cone subsampler (From Wrona et al., 1982).

6.4.2.5 Regardless of the method used for subsampling, the sorted sample should be labelled to reflect the portion sorted (e.g., 2X if half sorted, 4X if one-quarter sorted, 100 C if 100 count method was used, etc.) with the sample ID number. The unsorted portions of the sample should be combined, preserved, labeled and stored for future reference. It should be discarded only if there is no possible future need.

6.4.2.6 Experience has shown that, if less than one-quarter of the original sample is sorted, considerable error may result in estimating the total numbers of worms and other organisms that tend to clump. If the sample contains large numbers of a single taxonomic group (such as oligochaete worms

or midges) but few other organisms, it may be advisable to subsample the abundant taxa and pick all of the other organisms.

6.5 Preparation of Microscope Slide Mounts

6.5.1 To identify certain taxa of macroinvertebrates, it is often necessary to make slide mounts of all or parts of the organisms for examination under a compound microscope. Generally, if the organism is over 10 mm in length, it is best to carefully remove the important diagnostic structures (such as mouthparts or genitalia) with fine pointed forceps and mount them on microscope slides. Some large chironomids and tubificid worms that are too long to be mounted whole are cut in half and mounted under two separate cover glasses on the same slide.

6.5.2 Because most of the slides made for diagnostic purposes will be discarded after the organisms have been identified, we recommend mounting directly from the preservative using a water miscible mounting medium consisting of a mixture of two-thirds CMCP-9 and one-third CMCP-9AF (Beckett and Lewis, 1982). This mixture stains the organism a light red and contains a clearing agent providing optimum contrast for easy viewing of taxonomically important structures after about 12 hours clearing time. Because CMCP-9/9AF is a low viscosity medium, the specimen can be easily manipulated after the cover glass is in place by using pressure from forceps on the cover glass, rolling the specimen while viewing with a dissecting microscope until the best viewing position is obtained. The slides may be made permanent by ringing the cover glass with additional CMCP-9/9AF followed 24 hours later with polyurethane spar varnish or fingernail polish. Round 12 mm or 15mm cover glasses are recommended because they are less likely to trap air bubbles, are easier to manipulate, and less likely to break with pressure than the square ones. This method has proven very successful for making semi-permanent slides of whole chironomids and oligochaetes and parts of mayflies, caddisflies, and other macroinvertebrates.

6.5.3 Other slide-making techniques have been recommended for specific groups of organisms (Mason, 1973; Beck, 1975; Britton and Greeson, 1988). Although these methods are more time consuming and require more effort than the above method, they are thought to produce superior results by some taxonomists and are considered more permanent.

6.5.3.1 Many chironomid taxonomists use KOH to clear the midges before mounting them in Euparal (Mason, 1973) or CMCP-10 (Beck, 1975). The US Geological Survey (Britton and Greeson, 1988) has adopted a slightly modified version of this method for mounting midges and blackflies as follows:

1. Place the specimens in distilled water for 10 minutes to remove the preservative.
2. Transfer to crucibles containing 10% KOH and heat for 10 to 15 minutes to digest opaque tissue, taking care not to digest exoskeleton also.
3. Soak in distilled water for at least 3 minutes to remove KOH.
4. Soak in 95% ethyl alcohol for three to five minutes.
5. Mount in a drop of Euparal or CMCP-10.
6. Place specimen ventral side up and cover with a 12 mm cover glass.

7. Working under a stereoscopic microscope, apply pressure from a pencil eraser to roll ventral side up and flatten the head capsule.
8. Allow the slide to dry for about a week before storing on edge.

6.5.3.2 Water mites mounted using either of the above methods are nearly impossible to identify beyond family level. If identification to genus or species is needed, the mites should be dissected first to speed the clearing process and make it possible to examine sclerotized plates and other structures on both dorsal and ventral surfaces of the abdomen. First, using a dissecting microscope, forceps and a needle, separate one palp or the entire gnathostoma with palps from the body and mount the palps in the position shown in Figure 13. Next, separate the dorsum of the abdomen from the venter leaving a small section of the posterior body wall intact as shown in Figure 14, and mount with the venter and dorsum upward. Rather than dissect the very small specimens, pierce the body wall in the posterior-lateral areas to facilitate the clearing process and mount with the ventral surface upward (Britton and Greeson, 1988).

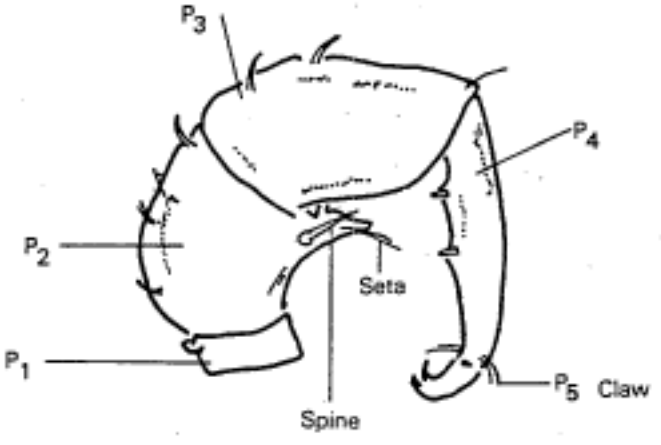

Figure 13. Five-segmented palp of a water mite (From Britton and Greeson, 1988).

6.5.3.3 When permanent slides are needed for the water mites, the double cover-glass glycerine method described by Mitchell and Cook (1952), modified by Britton and Greeson (1988), and illustrated in Figure 15 should be used.

6.5.4 Aquatic oligochaete worms--To identify oligochaete worms the specimens must be go through a clearing process and be side mounted. The identification of species requires a compound light microscope and some specimens require oil immersion (1000X). Some worm specialists make temporary mounts by placing oligochaete specimens on sides in Amman's lactophenol (100 g phenol, 100 ml lactic acid, 200 ml glycerine, 100 ml water), a medium which clears tissues and eliminates the risk of specimen desiccation if a more permanent

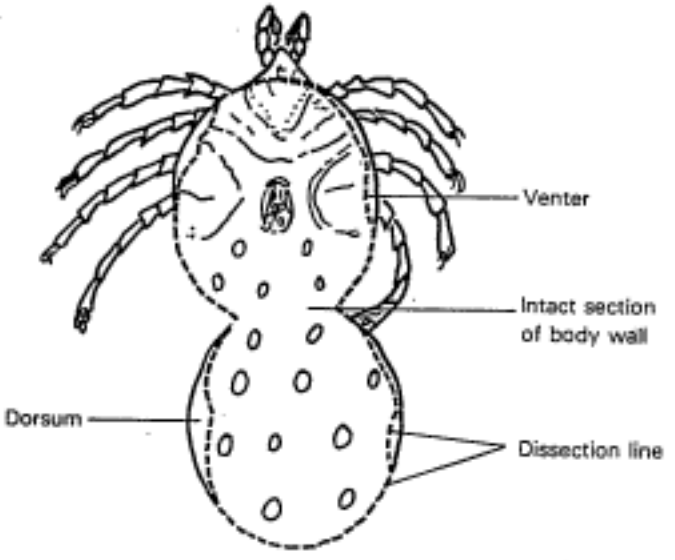

Figure 14. A water mite showing the dorsum separated from the venter, leaving a small section of the posterior body wall intact (From Britton and Greeson, 1988).

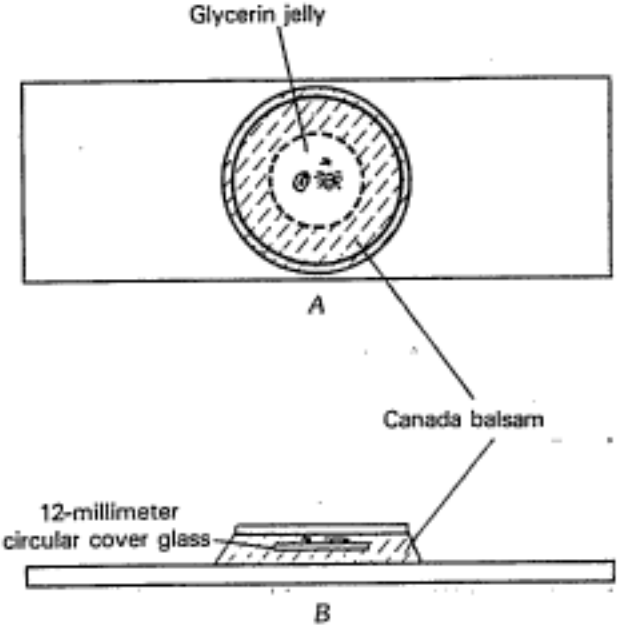

Figure 15. Top (A) and side (B) views of the double cover-glass technique for mounting aquatic water mites (From Britton and Greeson, 1988).

mount cannot be prepared immediately following extraction from the sample (Brinkhurst, 1986; Hiltunen and Klemm, 1980, Stimpson, et al., 1982; or Klemm, 1985). The clearing process usually takes a few hours to a few days depending on the size of the specimens. Gentle application of heat will speed the clearing process. If the specimens are preserved in 70-80% ethyl alcohol, they should be placed in 30% ethyl alcohol and then in water for a short time to leach out the alcohol before clearing. The alcohol retards the clearing process of Amman's lactophenol (Hiltunen and Klemm, 1980, Stimpson et al., 1982; Klemm, 1985). Do not leave specimens in the water too long (not more than two hours) because the worms will begin to deteriorate. Naidids and tubificids can be held indefinitely in Amman's lactophenol or 10% buffered formalin for later processing and mounting.

6.5.4.1 Non-resinous media are recommended for rapid processing of large numbers of specimens. For extremely important reference specimens, a permanent resinous mounting medium is best.

6.5.4.2 The non-resinous semi-permanent mounting media (CMCP-9 or 9AF, CMCP-10, or aquamount), which also contain clearing agents, are the simplest to use, allow for rapid processing of specimens, and are usually adequate for species identification. If Fuschin dye is added to the colorless mounting media (CMCP-9 or CMCP-10), only enough of the dye should be used (avoid overstaining) to slightly or partially stain the specimens. The specimens can be mounted directly on the slide using these media. However, the clearing process of these media takes approximately 24 hours. If the slides are to be semi-permanent, the edge of the cover slip should be sealed with finger nail lacquer to prevent the mounting medium from shrinking and forming bubbles under the cover slip. An 18 mm diameter, No. 0 or 1 round cover glass is appropriate because it will adequately accommodate the size range of the worms and the shape allows for maneuvering the specimen to rest in the most desired position by gentle rotation of the cover glass.

6.5.4.3 Place naidids or tubificids on their sides so that both dorsal and ventral fascicles of chaetae can be examined (Hiltunen and Klemm, 1980; Stimpson et al., 1982; Klemm, 1985). A variation from this is followed with specimens of Dero which must be viewed from the dorsal aspect, revealing the arrangement of the branchial apparatus (Hiltunen and Klemm, 1980, Klemm, 1985). The methods sections found in Hiltunen and Klemm (1980)and Klemm (1985) should be consulted for more specific information on identification of specimens.

6.5.4.4 Optimal resolution and longevity of mounted materials are achieved only in resinous media (e.g., Canada Balsam, Harleco's Xylene Coverbond, etc.). These mounting media require dehydration of the specimens through the alcohol series and clearing before mounting in Canada balsam or other resinous medium, but they produce the best permanent mounts (Knudsen, 1966; Klemm, 1985).

6.5.5 Leeches--species identification of most specimens do not require mounting on slides. A stereozoom microscope of 500X is needed for species identification. However, specialized slide-making techniques must be used for species identification of some leeches (See Klemm, 1982, 1985, 1990).

6.5.6 Regardless of the mounting method used or the permanence of the slides, proper labelling is a must. The label should include the date the slide was made, the sample ID number, and the initials of the person who made the slide. Labels on permanent slides should also include the location of the collecting site and name of the collector.

6.6 Drying Methods

6.6.1 Occasionally, alcohol-preserved specimens may require dry mounting on points or minutens for identification. The critical point drying method is recommended because the pigments colors are preserved, specimens do not collapse, and they are not brittle. Specimens to be dried are taken from 80% ethanol and passed through the alcohol series of washes in a small mesh screen basket with a lid, ending with two washes in 100% ethanol. After removal from the alcohol wash, the specimens, with the basket, are placed in the chamber of the critical point drier and processed according to dryer instructions (Gordh and Hall, 1979).

6.7 Organism Identification

6.7.1 The taxonomic level to which animals are identified depends on the needs, experience, and available resources. However, species level identification is very important in determining water quality and environmental pollution (Resh and Unzicker, 1975). The rapid bioassessment protocol II calls for organism identification only to the family level for use with Hilsenhoff's (1988) Family Biotic Index, whereas protocol III calls for identification to genus or species if possible (Plafkin et al., 1989). Many state programs carry most organism identifications to the genus level, while others (e.g., State of Maine) carry identification of certain taxa, such as stoneflies and mayflies, to species. Although the selective sensitivity of a family-level identification effort is often sufficient for differentiating non-impaired, moderately impaired, and severely impaired conditions, subtle differences in biological impairment will not be discerned except by species-level identification (Plafkin et al., 1989). In general, identifications should be carried to the lowest taxonomic level readily possible, and the taxonomic level to which identifications are carried in each major group should be constant throughout a given study.

6.7.1.1 Since the accuracy of identification depends on the availability of up-to-date taxonomic literature. A library of the basic taxonomic literature is essential for benthic laboratories. Basic references that should be available in a macroinvertebrate identification laboratory are listed in Section 8, Taxonomic Bibliography.

6.7.2 For comparative purposes and quality control checks, a reference collection of identified specimens should be established in each laboratory.

6.7.3 Most identifications to order and family can be made using a hand lens or a stereoscopic microscope with up to 50X magnification. Identification to genus and species often requires a compound microscope with phase contrast capable of 1000X magnification. Preparation of specimens for microscopic viewing is discussed in Section 6.5.

6.7.4 Insect larvae often comprise the majority of macroinvertebrates collected with artificial substrate samplers, drift nets, and other net type devices. In certain cases, identifications are facilitated if exuviae, pupae, and adults are available.

6.7.5 The life history stages of an insect can be positively associated only if specimens are reared individually. Small insect larvae can be reared individually in 6 to 12 dram vials half filled with stream water and aerated by use of a fine-drawn glass tubing. Mass rearing can be carried out by placing rocks and sticks containing the larvae in an aerated aquarium. Current can be provided in the aquarium by use of a magnetic stirrer (Mason and Lewis, 1970).

6.7.6 As organisms are identified, the individuals in each taxonomic category are counted and the numbers recorded on bench sheets (see Appendix C). Samples are compared by use of a summary sheet (see Appendix D) which provides room for comparing eight samples from the same sampling site.

6.8 Biomass

6.8.1 Macroinvertebrate biomass (weight of organisms per unit area) is a useful quantitative estimation of standing crop and is useful in assessing the biological integrity of surface waters. One study shows that biological assessments of water quality status using biomass estimates of wet, dry, and ash-free dry weights provide essentially similar results concerning impact of a sewage treatment plant discharge as did counts of individual organisms using a variety of commonly utilized biotic indices of water quality (Mason et al., 1983, 1985). To determine wet weights, soak the organisms in distilled or deionized water for 30 minutes, centrifuge for one minute at 140 g in wire mesh cones, and weigh to the nearest 0.1 mg. To obtain dry weight, dry the organisms to a constant weight at 105 degrees C for 4 hours or vacuum dry at 105 degrees C for 15 to 30 minutes at one-half atmosphere. Cool to room temperature for 15 minutes and weigh to nearest 0.1 mg. Freeze drying (-55 degrees C, 10 to 30 microns pressure) can be used. It has advantages over oven drying because the organisms remain intact for identification and reference, preservatives are not needed, and cooling the material in desiccators after drying is not required. The main disadvantage of freeze drying is the time (usually 24 hours) required for drying to a constant weight. To obtain ash-free dry weight, ash the dried organisms at 500 degrees C for one hour. Cool the ash to ambient temperature in a desiccator and weigh to the nearest 0.1 mg. Express the biomass as ash-free dry weight.

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